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1.	Your reference	~~REP07288GB~~ POFt/603 GB (All 27/6/03) 30 APR 2003
2.	Patent application number *(The Patent Office will fill in this part)*	0309927.2 01MAY03 E803998-2 D02890 P01/7700 0.00-0309927.2
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	Medical Biosystems Ltd. The Old Mill Beaston Cross Broadhempston Nr. Totnes Devon, TQ9 6BX
	Patents ADP number *(if you know it)*	7258635001
	If the applicant is a corporate body, give the country/state of its incorporation	GB
4.	Title of the invention	BIOSENSOR
5.	Name of your agent *(if you have one)*	Gill Jennings & Every
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	Broadgate House 7 Eldon Street London EC2M 7LH
	Patents ADP number *(if you know it)*	745002
6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country / Priority application number *(if you know it)* / Date of filing *(day / month / year)*
7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application / Date of filing *(day / month / year)*
8.	Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if: a) any applicant named in part 3 is not an inventor, or b) there is an inventor who is not named as an applicant, or c) any named applicant is a corporate body. See note (d))*	YES

Patents Form 1/77

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Continuation sheets of this form	0
Description	6
Claim *(s)*	4
Abstract	0
Drawing *(s)*	3 + 3 JMC

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	NO

11. For the applicant
Gill Jennings & Every

I/We request the grant of a patent on the basis of this application.

Signature: Gill Jennings & Every

Date: 30 April 2003

12. Name and daytime telephone number of person to contact in the United Kingdom

Mr John Jappy
020 7377 1377

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DUPLICATE

BIOSENSOR

Field of the Invention

The present invention relates to a system for detecting a physical, chemical or biochemical reactions, and in particular to a system in which surface electromagnetic waves (SEWs) interact with a specimen involved in the reaction.

Background to the Invention

Biosensors incorporating surface electromagnetic wave technology are well known. In particular surface plasmon resonance (SPR) sensor technology is a leading technology in the field of real time observation of bimolecular reactions. However, all of the systems disclosed in the prior art suffer from problems, and in particular suffer from sensitivity to vibration and to variation in the refractive index of the medium in which the reaction takes place caused by temperature fluctuations.

Summary of the Invention

According to a first aspect of the present invention a system for detecting a physical, chemical or biochemical reaction comprises:

a coherent radiation source for producing an incident wave;

a carrier surface for supporting a specimen to be analysed, the carrier surface mounted on a substrate and capable of supporting surface electromagnetic waves (SEW);

means for splitting the incident wave into an SEW and a first scattered wave, wherein the SEW propagates along the carrier surface and interacts with the specimen;

means for generating a second scattered wave from the SEW; and,

a detector for monitoring the interference between the first scattered wave and the second scattered wave.

According to a second aspect of the present invention a carrier chip for a specimen to be monitored, comprises:

a dielectric substrate; and

a conductive film formed on the surface of the substrate suitable for carrying the specimen;

wherein the conductive film comprises first means for splitting an incident wave into a first scattered wave and a surface electromagnetic wave (SEW), the SEW propagating along the carrier surface and interacting with the specimen, and a second means for generating a second scattered wave from the SEW.

According to a third aspect of the present invention a method of monitoring a specimen undergoing a physical, chemical or biochemical reaction occurring on a surface supporting surface electromagnetic waves (SEW), comprises the steps of:

splitting an incident wave into a first scattered wave and SEW such that the SEW propagates along the surface and interacts with the specimen;

splitting the SEW which has interacted with the specimen to generate a second scattered wave; and,

monitoring the interference pattern between the first and second scattered waves.

Brief Description of the Drawings

Examples of the present invention will now be described with reference to the accompanying drawings in which:

Figure 1 is a schematic illustration of an apparatus according to the present invention for detecting a physical, chemical or biochemical reaction;

Figure 2 illustrates a second embodiment of a system for detecting a physical, chemical or biochemical reaction;

Figure 3 shows detail of a carrier chip in accordance with the present invention; and

Figure 4 shows detail of a second carrier chip in accordance with the present invention.

Detailed Description

Figure 1 shows a system for monitoring a physical, chemical or biochemical reaction in accordance with the first embodiment of the present invention. A coherent optical beam generated by a monochromatic laser is focused using a lens, onto the edge of a metallic film able to support surface electromagnetic waves (SEWs). The optical beam passes through the glass prism on which the metallic film is mounted. The prism may be a triangular prism as shown, or more preferably a cylindrical prism. The metallic film is formed from gold (or alternatively

from silver) and is deposited on the prism using a lithographic process. The thickness of the gold or silver film is such that it allows SEWs i.e. surface plasmons, to be exited on its surface.

The right hand edge of the metallic film causes the incident optical beam to scatter. Radiation is scattered over a range of angles, including along the surface of the film. Scattered waves on the surface of the film interact with the free electrons in the carrier surface to form surface plasmons. Radiation scattered through other angles form volume or optical radiation.

The surface plasmons travel along the surface of the film until they reach the opposite edge. At the opposite edge the plasmons are scattered and generate further optical waves. Since a coherent light source is used the optical waves generated at the first edge of the film are coherent to the optical waves generated at the second edge. These two sets of optical waves will interfere to form an interference pattern, the properties of which depend on the optical path length difference between the interfering waves. The optical path length is dependent both on the absolute length that the waves travel, as well as the effective refractive index that they experience.

The effective refractive index experienced by the surface plasmons is dependent on a number of factors including the refractive index of the reaction fluid within the reaction vessel, the refractive index of the prism supporting the film and the charge distribution of any specimens mounted on the carrier film. The charge distribution affects the effective refractive index and hence the phase velocity of the surface plasmon. By calibrating the apparatus when there are no specimens on the film (and hence no reaction taking place) the change in the phase velocity of the surface plasmons effected by the reaction can be determined. In particular, the apparatus is suitable for detecting the generation of the complimentary base pairs in a strand of DNA. A complimentary DNA strand can be produced using a polymerase and a "parent" DNA strand. A DNA strand is built from four base blocks, and binding of each of these four blocks to a DNA strand will lead to a characteristic charge distribution in a polymerase on the surface of the film. This in turn will lead to a characteristic change in the phase velocity of the SEW and hence a characteristic change in the interference pattern. The use of surface plasmon resonance in the detection of nucleotide incorporation during DNA

synthesis, is disclosed in WO-A-99/05315, the content of which is hereby incorporated by reference.

In order to increase accuracy a number of identical specimens can be placed along the length of the film so as to give rise to a greater interaction length between the specimen and the plasmons. Characteristic phase changes for particular reactions can be found by monitoring known reactions under known conditions.

There are a number of detectors which can be used to detect the interference pattern generated by the scattered waves from both ends of the film. As shown in Figure 1 a 2-section photodiode is used but other sensors such as a CCD array could be used.

As shown in Figure 1, the reaction which is being monitored takes place inside a reaction vessel which contains a liquid medium. The refractive index of this medium is dependent on the ambient conditions and in particular is dependent upon the ambient temperature. Any variation in the ambient temperature will therefore change the refractive index and hence change the optical path length experienced by each of the interfering waves. However, in contrast to prior devices, the apparatus shown in Figure 1 is set up such that both the interfering waves pass through the liquid medium in the reaction vessel. This means that, to a large extent, variations in the refractive index of the liquid will not effect the interference pattern because both paths will be affected. It can also be seen that the apparatus shown in Figure 1 is not sensitive to vibrations to the metallic film, as both interfering waves are generated from the metallic film. In prior devices only one of the interfering beams is incident on the metallic film, the reference beam passing outside the reaction vessel, so that vibrations of the metallic film cause large errors.

Figure 2 shows an improved set up to that shown in Figure 1. As shown in Figure 2 the reaction vessel is shaped so as to minimise the effect of a change in the refractive index of the liquid in the reaction vessel on the interference pattern detected by the detector. The angle of the exit face of the reaction vessel relative to the film can be optimised by minimising an expression which determines the variation of the angle at which an interference maximum is experienced with variation in refractive index of the reaction fluid. Detail of the mathematics behind the design and the degree of improvement of the apparatus shown in Figure 1 and

in Figure 2 as compared with prior designs can be found in co-pending UK patent application number GB 0220341.2.

Figure 3 shows a close up of the profile of a film for use in the apparatus of Figure 1 or Figure 2 in accordance with another aspect of the present invention. As shown in Figure 3 a carrier film is mounted on the surface of a supporting prism made of a transparent dielectric material. The carrier film includes a first section 31 of a first thickness and a second section 32 of greater thickness. Coherent radiation 33 incident on the edge of the first section will scatter and will generate a SEW 34 on the surface as described with reference to Figure 1. This SEW 34 will propagate along section 31 of the carrier film until it reaches the boundary between sections 31 and 32. At this boundary the SEW will again scatter generating a further SEW 35 which propagates along section 32 on which a specimen or specimens to be monitored is mounted and will also generate volume radiation 36 or optical waves which will scatter out into the reaction medium. The SEW propagating along section 32 of the carrier film will reach the right hand edge of section 32 and again will scatter, generating further volume radiation 37 and this volume radiation will interfere with the volume radiation scattered from the left hand edge of the carrier film section 32 to form an interference pattern. This interference pattern will be indicative of the reaction taking place on the surface of the carrier film as explained with reference to Figures 1 and 2. The film illustrated in Figure 3 uses a surface electromagnetic wave 34 to generate both the interfering waves which form the interference pattern, whereas in Figures 1 and 2 the film is designed such that the first scattered wave of interference pattern is generated directly from an incident laser beam.

Figure 4 shows a further design modification to the film. Figure 4 shows a film with a first section 41 on which the specimen or specimens to be monitored are mounted and, a second section 42 of different thickness to the first section. With the design shown in Figure 4 a first scattered wave 43 is generated at the left hand edge of the section 41 of the film and a second scattered wave 44 is generated at the right hand edge of the section 41 of them film, with a characteristic optical path length difference between the two waves indicative of the reaction taking place on the surface of the film. The second section of the film 42, which is on the right of the first section 41, covers the surface of the supporting prism to ensure that no

external light enters the apparatus which might affect the quality or resolution of the interference patent generated by the scattered waves.

Further film designs are possible, incorporating the features of both Figures 3 and 4. It is also possible to induce scattering from the surface of the film by introducing a change in the refractive index of the film or surrounding materials at the point at which scattered waves are to be generated.

It is also envisaged that the interference between two SEWs could be monitored, with the first SEW interacting with the specimen mounted on the carrier surface and a second SEW not interacting with the sample. Plasmon circuitry to this end could be formed lithographically. In this way, a surface plasmon interferometer could be used to monitor the physical, chemical or biochemical reaction taking place on the film. This type of system would also be less sensitive to variation in the refractive index of the reaction fluid and to vibrations than prior designs.

CLAIMS

1. A system for detecting a physical, chemical or biochemical reaction comprising:

a coherent radiation source for producing an incident wave;

a carrier surface for supporting a specimen to be analysed, the carrier surface mounted on a substrate and capable of supporting surface electromagnetic waves (SEW);

means for splitting the incident wave into an SEW and a first scattered wave, wherein the SEW propagates along the carrier surface and interacts with the specimen;

means for generating a second scattered wave from the SEW; and,

a detector for monitoring the interference between the first scattered wave and the second scattered wave.

2. A system according to claim 1, wherein the incident wave is a SEW.

3. A system according to claim 1 or 2, wherein the means for splitting the incident wave is a discontinuity in the carrier surface.

4. A system according to any preceding claim, wherein the means for generating the second scattered wave is a discontinuity in the carrier surface.

5. A system according to claim 3 or 4, wherein the discontinuity is a discontinuity in the thickness of the carrier surface.

6. A system according to claim 3 or 4, wherein the discontinuity is a discontinuity in the refractive index of the carrier surface or adjacent materials.

7. A system according to any preceding claim, wherein the specimen is contained in a reaction vessel containing a reaction fluid, and wherein both the first and second scattered waves pass through the reaction fluid.

8. A system according to claim 7, wherein the detector is positioned outside the reaction vessel.

9. A system according to claim 8, wherein the reaction vessel is shaped relative to the position of the carrier surface and the position of the detector so as to minimise the effect of fluctuation in the refractive index of the reaction fluid on the interference detected by the detector.

10. A system according to any preceding claim, wherein the SEW is a surface plasmon.

11. A system according to any preceding claim, further comprising a polymerase on the carrier surface suitable for matching complimentary base pairs of a DNA strand, wherein the system is used to monitor a DNA sequencing operation.

12. A carrier chip for a specimen to be monitored, comprising:

a dielectric substrate; and

a conductive film formed on the surface of the substrate suitable for carrying the specimen;

wherein the conductive film comprises first means for splitting an incident wave into a first scattered wave and a surface electromagnetic wave (SEW), the SEW propagating along the carrier surface and interacting with the specimen, and a second means for generating a second scattered wave from the SEW.

13. A carrier surface according to claim 12, wherein the first and second means are discontinuities in the conductive film.

14. A carrier surface according to claim 13, wherein the first or second means is a discontinuity in the thickness of the conductor film.

15. A carrier surface according to claim 13, wherein the discontinuity is a discontinuity in the refractive index of the carrier surface or adjucent materials.

16. A carrier surface according to claim 12, wherein the second means is an increase in the thickness of the film in the direction of propagation of the SEW.

17. A method of monitoring a specimen undergoing a physical, chemical or biochemical reaction occurring on a surface supporting surface electromagnetic waves (SEW), comprising the steps of:

splitting an incident wave into a first scattered wave and SEW such that the SEW propagates along the surface and interacts with the specimen;

splitting the SEW which has interacted with the specimen to generate a second scattered wave; and,

monitoring the interference pattern between the first and second scattered waves.

18. A method according to claim 17, wherein the incident wave is a SEW.

19. A method according to claim 17 or 18, wherein the incident wave is generated by a coherent light source.

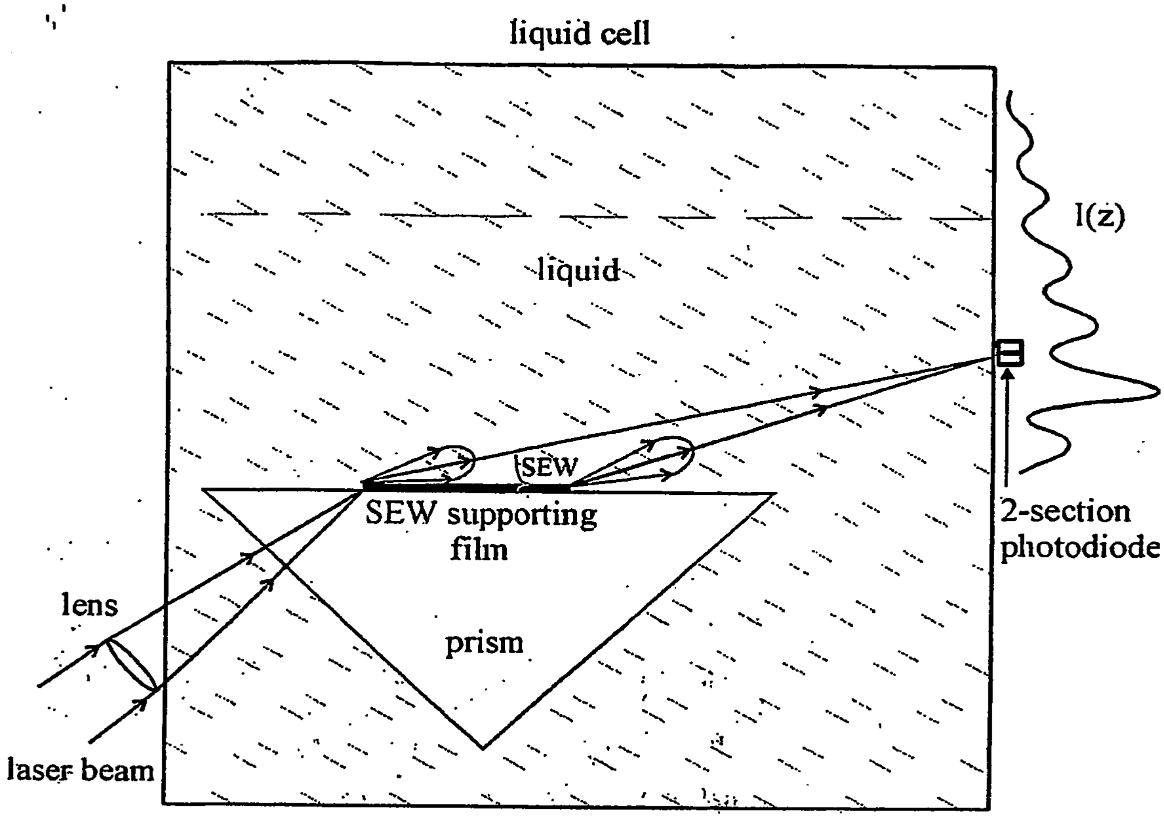
20. A method according to any one of claims 17 to 19, wherein the specimen is held within a reaction fluid in a reaction vessel, and the first and second scattered waves both pass through the reaction fluid.

21. A method according to claim 20, wherein the monitoring of the interference pattern takes place outside of the reaction vessel.

22. A method according to claim 21, wherein the reaction vessel is shaped so as to minimise the effect of fluctuations in the refractive index of the reaction fluid on the interference pattern between the first and second scattered waves.

23. A method according to any one of claims 17 to 22, wherein the specimen includes a polymerase, and the SEW interacts with the polymerase as it incorporates nucleotides into a polynucleotide strand complementary to a target polynucleotide.

24. A carrier surface according to any one of claims 12 to 16, together with an immobilised polymerase enzyme fixed to the carrier surface.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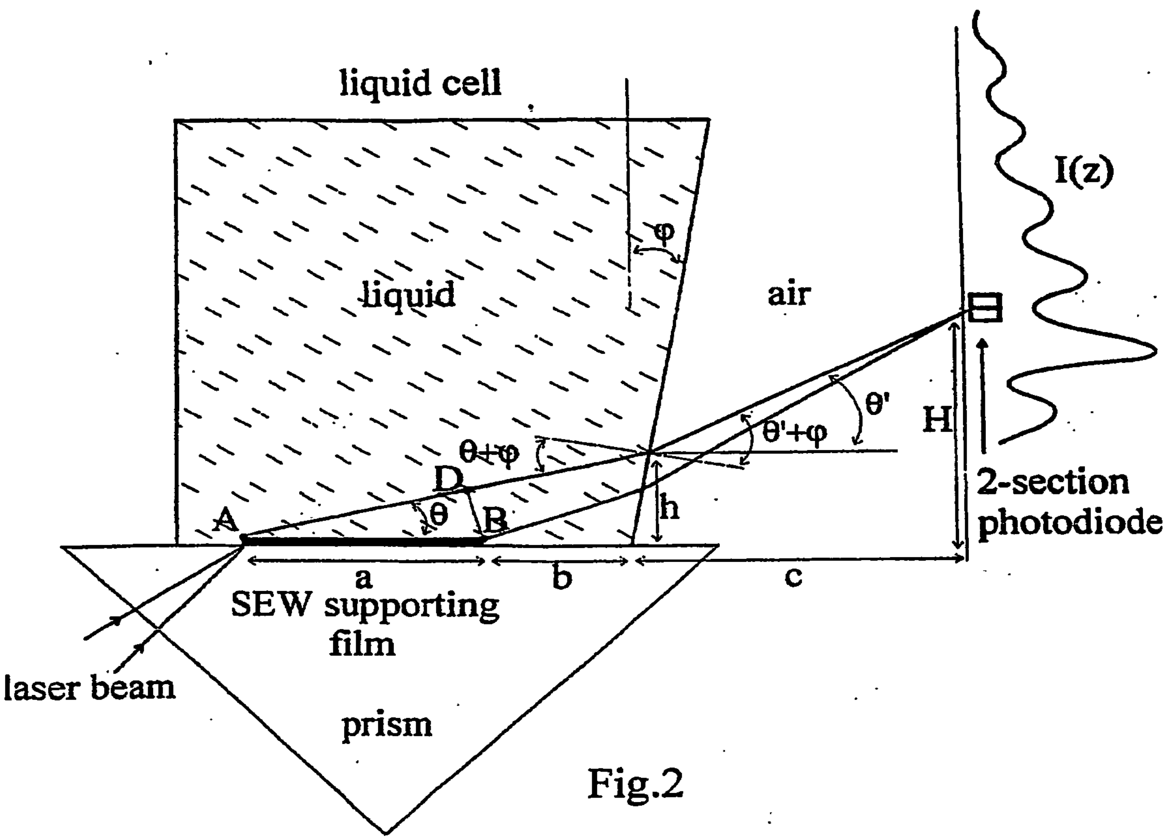
I(z)
liquid
SEW
SEW supporting film
2-section photodiode
lens
prism
laser beam

Fig.1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c
SEW supporting
film
laser beam
prism

Fig.2

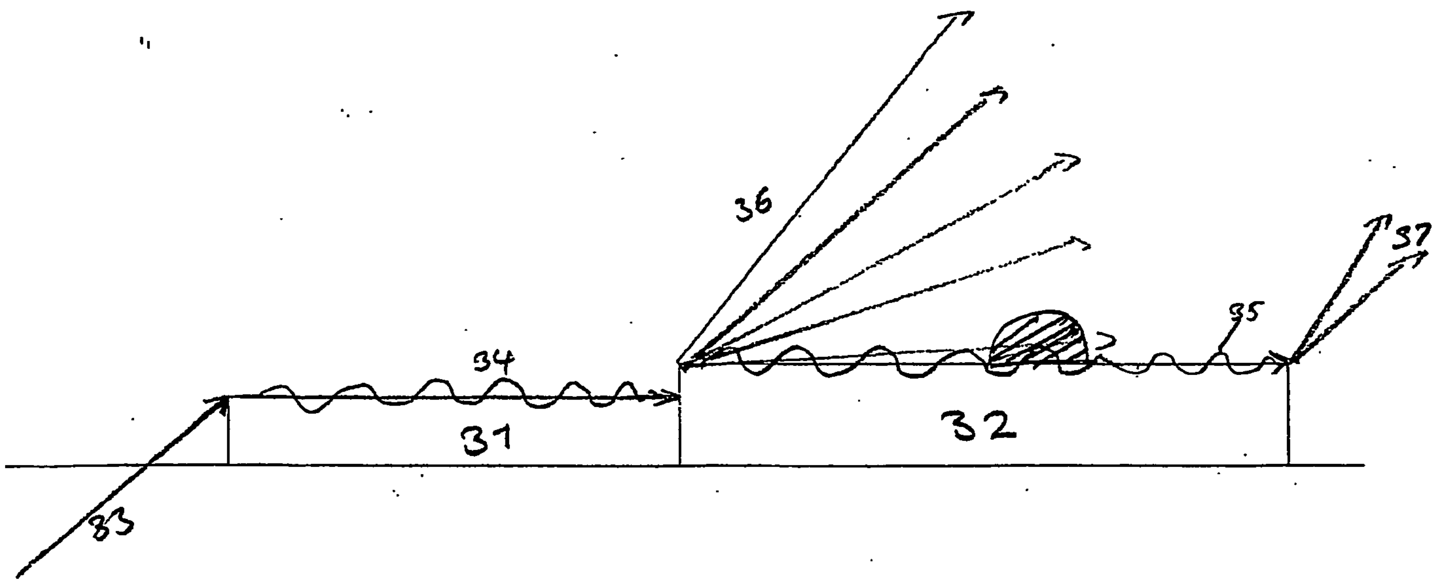

Fig. 3

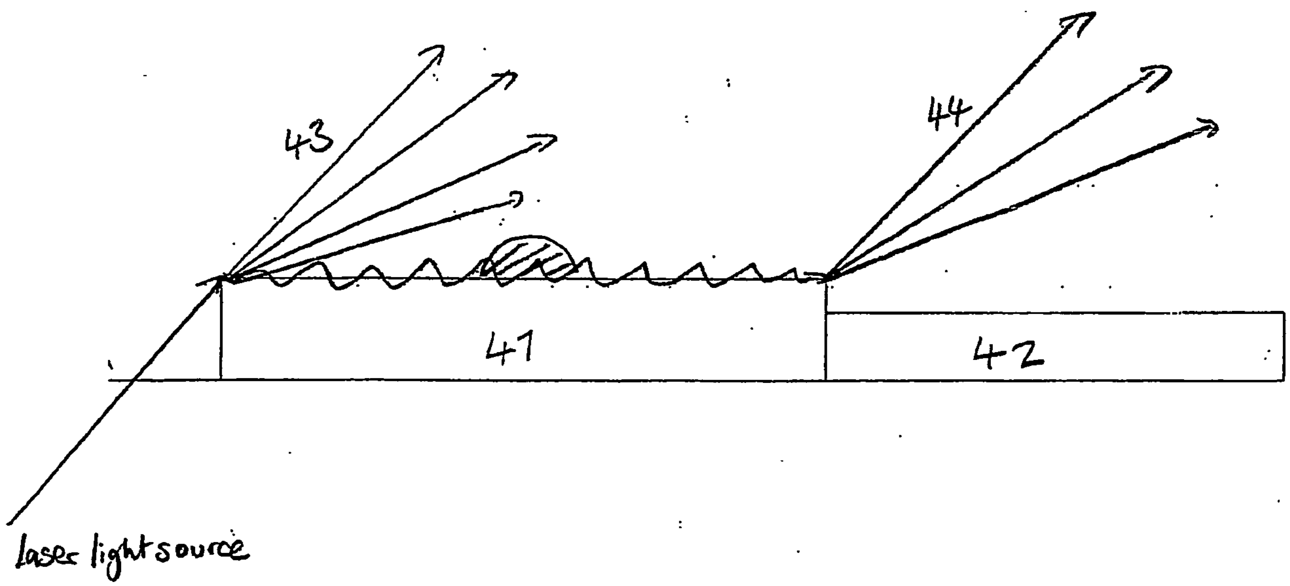

Fig.4